

EFFECT OF IONIZING RADIATION ON PROSTAGLANDINS AND GASTRIC SECRETION
IN RHESUS MONKEYS

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Research was conducted according to the principles enunciated in the
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Abstract

DUBOIS, A., DORVAL E.D., FIALA N., AND CONKLIN J.J.. Effect of Ionizing Radiation on Prostaglandins and Gastric Secretion in Monkeys. Rad Res

The radiation induced prodromal syndrome is characterized by nausea and vomiting. We have previously shown that gastric emptying, gastric motility, and gastric secretion were suppressed after total body exposure to irradiation. In the present studies, we evaluated the relation between vomiting and gastric function, ^{is evaluated} and ~~we explored~~ ^{is explored} the possible role of prostaglandins (PG) in these phenomena. ~~We determined~~ ^{was determined} the concentration of PG in the plasma and gastric juice ^{was concurrently measured} using standard radioimmunoassay and concurrently measured gastric acid output using a marker dilution technique in 9 rhesus monkeys. The animals were studied in the basal state and after total body exposure to 800 cGy Cobalt-60 delivered at a rate of 500 cGy/min. Acid output was abolished from 40 min to 2 hrs after irradiation but had returned to preirradiation levels 2 days later. Plasma PGF_2 and PGI_2 (as measured by 6 keto- $\text{PGF}_{1\alpha}$ determination) were not significantly modified by irradiation. In contrast, irradiation produced an immediate increase ($p < 0.05$) in gastric juice concentration of PGE_2 (318 \pm 80 to 523 \pm 94 pg/ml; mean \pm SE) and PGI_2 (230 \pm 36 to 346 \pm 57 pg/ml); Both had returned to basal levels 2 days later. Thus, both PGE_2 and PGI_2 may be responsible for the immediate suppression of acid output. Furthermore, our observations suggest that measurement of PG concentration in the gastric juice is useful to examine the role of prostaglandins in gastric function.

Introduction

Emesis is the most obvious and the best documented prodromal symptom of the acute radiation sickness which occurs immediately after whole body irradiation (1,2). We and others have recently shown that these symptoms are accompanied by a suppression of gastric emptying and gastric secretion (3-5). This radiation induced prodromal syndrome is clearly different from the condition observed during the intestinal syndrome which occurs 7 to 15 days after irradiation and is characterized by bloody diarrhea (6).

The chemoreceptor trigger zone and the vomiting center in the brain appear to play a pivotal role in irradiation-induced vomiting (7-9), but the role of peripheral mediators is not yet completely elucidated, and the mechanism of the suppression of gastric emptying and secretion remains unclear. Prostaglandins could be involved as a paracrine mediator released peripherally and acting directly on the gastrointestinal tract, as they can produce vomiting (10-12) and their concentrations are increased in the small bowel of the rat after irradiation (13).

The present studies were performed in monkeys, an animal model which appears closest to man in term of brain organization and gastric function. We produced vomiting and gastric suppression with a single dose of total body irradiation and we measured prostaglandins and gastric function before, during and after the acute radiation sickness. We determined the concentration of prostaglandins E_2 (PGE_2) and 6-keto-Prostaglandin $F_{1\alpha}$ (a stable metabolite of PGI_2 (14,15); 6-keto- $PGF_{1\alpha}$) in the plasma and in the gastric juice. In addition, we examined the relation between these prostaglandin levels and radiation-induced vomiting and stomach function.

Materials and Methods

Nine conscious, chair-adapted rhesus monkeys were studied on 3 separate days after an overnight fast: control day, irradiation day and 2 days after irradiation. Studies were performed in the morning and started 20 min after either sham irradiation on control day, or after real irradiation on irradiation day. On control days, the animals were brought to the exposure room and the doors were closed for 3 min. On irradiation day, each monkey was exposed to 800 cGy (800 rads) total body irradiation delivered at 500 cGy/min by two large, 10^5 Ci ^{60}Co Cobalt irradiators placed anteriorly and posteriorly. Phantom studies demonstrated that the midline abdomen received 800 cGy and that the head received 600 cGy.

Each monkey was visually monitored for 3 hours on control days and for 6 hours on irradiation days. In addition, bipolar electrical potentials were recorded from 2 abdominal disposable skin electrodes on a multichannel recorder (Beckman R612, Beckman Instr., Schiller Park, IL). Abdominal bipolar recordings displayed periodic waves in the 3/min range which have been shown to correlate with gastric electrical control activity when gastric serosal electrodes are used in conjunction with skin electrodes (16,17). Each tracing was examined blindly by one of us (EDD), the total duration during which gastric waves could be counted was determined, and the mean gastric frequency was determined by visual inspection as previously reported (5). Vomiting was defined as a succession of strong and brief contractions of thoracic and abdominal muscles leading to the expulsion of gastric contents through the mouth; retching was defined as a non-productive vomiting (18). During both events, recordings displayed a succession of brief bursts of high potential spikes (5), clearly different from the movement artifacts which were sometimes

superimposed.

A 12 French double lumen nasogastric tube was placed in the stomach, and a sample of gastric juice was obtained for measurement of prostaglandins. The water recovery test (19) was then used to verify the position of the tube, which was satisfactory in all experiments. Starting 45 min later, a previously described and validated marker dilution technique (20,21) was used concurrently to calculate gastric secretion and gastric emptying as previously reported (5). This procedure was performed once a week for 1 month in order to habituate the monkeys before initiation of the studies. The subsequent studies were performed with the same frequency.

Before each of the studies, blood was drawn and placed in tubes containing 2% EDTA (0.08 mg/ml blood) and 1.6% solution of indomethacin in NaHCO_3 buffer (0.005 mg/ml blood) for determination of levels of 6-keto-PGF_{1 α} (a stable metabolite of PGI₂), and PGE₂ in the plasma. Samples of gastric juice were placed in tubes containing 1.6% solution of indomethacin in NaHCO_3 buffer (0.005 mg/ml). Samples of plasma and gastric juice were immediately centrifuged and the clear supernatants were frozen at -80 C for subsequent analysis. At the time of the analysis, samples were thawed and then applied to individual C18 solid phase extraction columns (Bone-Elut C18, 200 mg sorbent mass, Analytichem International, Harbor City, CA) previously washed with methanol and equilibrated with H₂O adjusted to pH 3.2 with citric acid. When the supernatants had passed through the columns, each was sequentially washed with 2 ml of H₂O (pH 3.2), 2 ml 12.5% methanol and 2 ml benzene. Prostaglandins were eluted off the C18 column with four 0.5 ml aliquots of ethyl acetate. Slight vacuum pressure was applied to capture the remaining solvent from the columns. Ethyl acetate extracts were dried under N₂ and subsequently reconstituted in assay buffer (phosphate buffered saline pH 6.8

containing 1% bovine gamma-globulin and 0.05% sodium azide). Prostaglandin E_2 and 6-keto-PGF $_{1\alpha}$ levels were determined by radioimmunoassay, employing ^{125}I -radioimmunoassay kits (New England Nuclear, Boston, MA).

Samples of plasma and gastric juice (1.0 ml) were acidified to pH 3.2 by the addition of approximately 0.1 ml 2 M citric acid. Centrifugation for 10 min at 100 x g (4 C) did not result in a precipitable protein pellet. Acidified samples were applied to individual C18 extraction columns (500 mg sorbent mass), sequentially washed and PGs recovered from the column as previously described. Following N_2 evaporation of the ethyl acetate, extracts were reconstituted in 0.1ml assay buffer and PG levels determined by radioimmunoassay as previously described (22).

Representative samples of plasma and gastric juice were spiked with 10,000 CPM 3H -PGE $_2$ or 3H -6-keto-PGF $_{1\alpha}$ and processed with other samples, in order to determine extraction efficiency. Recovery of all four PG tracers was greater than 95% in plasma samples and greater than 90% in biopsy samples. Similarly, samples were analyzed for non-specific interfering materials. Samples of plasma and gastric juice were depleted of PGs by charcoal adsorption (Norit A, Fisher Chemical Co., Fairlawn, NJ) (22). One ml plasma samples and 3 ml gastric juice samples were incubated for 2 hr at 4 C with 0.15 or 0.45 ml, respectively, of a 100 mg/ml charcoal-saline solution. (Tracer amounts of labeled PGs added to the samples revealed this procedure to completely adsorb the radioactivity.) PG-depleted plasmas and gastric juices were processed on C18 extraction columns as described. Evaporated ethyl acetate eluates, reconstituted in assay buffer and analyzed by radioimmunoassay, revealed less than 5% decrease in binding of all three radiolabeled ligands to their respective antibodies. A number of plasma and gastric juice PG extracts were analyzed at two dilutions. The results, when

corrected for the dilution factor, yielded sample values within 10% of one another.

All calculations were performed using locally developed programs and a PDP-10 computer (Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland). The statistical significance of differences observed for each measurement of prostaglandin concentrations and gastric function (i.e., fractional emptying rate, acid output, etc...) was evaluated using a three-factor (treatment, time, and monkey) analysis of variance with repeated measures on the last two factors (20,21), the program LDU-040 (K.L. Dorn), and an IBM 370 computer (Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD).

Results

Two hours after irradiation, plasma PGE_2 and 6-keto- $\text{PGF}_{1\alpha}$ were not significantly different from control day or from 2 days post irradiation (table 1).

In contrast, a significant increase ($p < 0.05$) of the concentration of PGE_2 and PGI_2 was observed in the gastric juice 30 min after irradiation; both concentrations had returned to basal levels 2 days later (table 2).

The increase of the concentration of PGE_2 and PGI_2 in the gastric juice occurred concurrently with radiation induced vomiting, suppression of acid output, and suppression of gastric motility as previously reported (5). However, there was no significant correlation between gastric acid output, gastric emptying, and gastric motility on one hand, and the concentration of PGE_2 or PGI_2 in the gastric juice on the other hand.

Discussion

The present studies demonstrate that, in monkeys, total body irradiation produces an increase of the concentration of PGE_2 and PGI_2 in the gastric juice in monkeys. This increase occurs concurrently with the vomiting, suppression of acid output, suppression of gastric motility (5) and increase of gastric mucus production (23,24) that are observed after exposure to irradiation in the same animal model. Therefore, it is tempting to propose that the local release of PG is responsible for the gastric effects of radiation. This hypothesis is supported by the observation that exogenous administration of sufficient doses of prostaglandins produce vomiting (10-12), inhibits gastric acid secretion (10,25-28), and stimulate gastric mucus output (29). In addition, since gastric emptying is increased by PGE_2 (26) and decreased by PGI_2 (27), PGI_2 may be responsible for the suppression of gastric emptying. However, the absence of correlation between the concentration of PG in the gastric juice and the gastric parameters suggests that additional factors play a role in the gastric effects of irradiation.

In contrast to the clear effect of radiation on the concentration of PG in the gastric juice, plasma PGE_2 and PGI_2 remained unchanged during vomiting and suppression of gastric secretion and emptying. This observation agrees with absence of changes in plasma PG reported in human radiation-induced enteritis (30). It could mean that these circulating PG are not involved either in radiation-induced emesis or in the suppression secretion and emptying. This, however, would not exclude that the effect of radiation is mediated by a local release of gastrointestinal PG which would not be reflected by circulating PG. Alternatively, an increase of plasma PG could have occurred during or immediately after irradiation, and could have

initiated vomiting and/or suppression of gastric functions but is no longer detectable two hours post irradiation because of the short half life of PG in the plasma (27, 31).

The exact mechanisms of gastric secretion and emptying suppression (e.g., local gastric release of PG) is not clarified by our study. However, the time of onset of each symptom after irradiation and the transiency of the acute radiation syndrome appears to exclude a direct damage to gastric mucosal cells, to the myenteric plexus, or the smooth muscle similar to the one observed during the late postirradiation period (5).

In conclusion, a local release of PGE_2 and PGI_2 may play a role in the suppression of gastric function and the emetic episodes observed immediately after irradiation. Furthermore, our observations suggest that measurement of PG concentration in the gastric juice is useful to examine the role of prostaglandins in gastric function.

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Table 1 - Effects of irradiation on plasma prostaglandins (pg/ml;
means \pm SE).

| | Irradiation Day | | |
|--|--------------------|--------------------|------------------------------|
| | 2 Hr after | | |
| | <u>Control Day</u> | <u>Irradiation</u> | <u>2 Days Post Radiation</u> |
| PGE ₂ | 425.4 \pm 29.4 | 459.8 \pm 38.4 | 397.3 \pm 15.5 |
| | N=9 | N=8 | N=8 |
| 6-Keto-PGF _{1α} | 415.0 \pm 59.0 | 469.1 \pm 44.5 | 379.9 \pm 27.1 |
| | N=10 | N=9 | N=10 |

Table 2. Effect of irradiation on the concentration of PG in the gastric juice (pg/ml; means \pm SE; * $p < 0.05$ compared to control).

| | <u>Control Day</u> | <u>Irradiation Day</u> | 2 Days Post <u>Irradiation</u> |
|--|--------------------|------------------------|-----------------------------------|
| PGE ₂ | 318 \pm 80 | 523 \pm 94* | 305 \pm 65 |
| 6-Keto-PGF _{1α} | 230 \pm 36 | 346 \pm 57* | 320 \pm 40 |

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